CHEMOPREVENTIVE EFFECT OF TOPICAL APPLICATION OF S-ALLYLCYSTEINE IN THE MANAGEMENT OF ORAL DYSPLASTIC POTENTIALLY MALIGNANT DISORDERS

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ABSTRACT

INTRODUCTION: Oral potentially malignant disorders (OPMDs) describe mucosal disorders with an increased risk of malignant transformation (MT) to oral squamous cell carcinoma (OSCC). Natural products like garlic represent a promising group of chemopreventive agents. Aged garlic extract (AGE) is one of the most commonly used garlic preparations as it contains more stable organosulfur compounds (OSCs); S-allylcysteine (SAC) is the most abundant organosulfur compound in AGE. SAC has been found to retard the growth of chemically induced and transplantable tumors in several animal models.

OBJECTIVES: To evaluate the cancer chemopreventive effect of topically applied S-Allylcysteine in the management of oral dysplastic potentially malignant disorders.

MATERIALS AND METHODS:10 subjects with oral dysplastic potentially malignant disorders, as proven clinically and histopathologically, were recruited for this study. They received topical S-Allylcysteine for 1 month, and then the lesions were evaluated both clinically and histopathologically after termination of therapy to assess any alterations in the lesions' size, pain score and mucosal dysplasia.

RESULTS: S-allylcysteine was well tolerated by all the patients. After termination of the therapeutic phase (after one month), S-Allylcysteine was found to decrease the pain score in all symptomatic patients. The size of the lesions was also decreased although it was not statistically significant; however, histological improvement was remarkable. Complete histological response was observed in four leukoplakia patients and two lichen planus patients where the mild and moderate dysplastic changes showed histologic remission of dysplasia. However, two leukoplakia cases showed progression in the grade of dysplasia from mild to moderate.

CONCLUSIONS: Topical application of S-Allylcysteine is beneficial for the management of dysplasia associated with oral potentially malignant disorders**.**

KEYWORDS: Oral potentially malignant disorders, garlic, S-allylcysteine**,** cancer chemoprevention**,** dysplasia.

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INTRODUCTION

Cancer of the oral cavity accounts for over 2% of all malignancies and is found to affect about 300,50 patients annually worldwide (1). Oral squamous cell carcinoma (OSCC) comprises 92–95% of all oral cancers (2). Many OSCCs develop from oral potentially malignant disorders (PMDs) which supports the concept of two-step cancer development process (3).

Oral potentially malignant disorders (OPMDs) include a variety of lesions and conditions characterized by an increased risk for malignant transformation (MT) to oral squamous cell carcinoma (OSCC). They include erythroplakia, leukoplakia, oral submucous fibrosis, lichen planus, along with inherited syndromes like xeroderma pigmentosum and Fanconi's anemia (4).

The histopathological features of a given lesion, especially the presence and degree of epithelial dysplasia, are currently accepted as the most useful indicators of MT risk (5). Severe epithelial dysplasia was found to have a malignant transformation rate of about 7–50%, whereas mild epithelial dysplasia shows a low risk (<5%) (6).

Excision is now generally accepted to be the treatment of choice for dysplastic OPMDs, however it still carries a high risk of recurrence (up to 35%). Furthermore, resection of large lesions can cause significant morbidity and sometimes require extensive reconstructive techniques (7). Therefore, nonsurgical treatment is considered strongly for the management of PMDs. This modality offers many advantages including ease of application, relative low cost, in addition to minimal adverse effects to the patients especially for those with widespread involvement of the oral mucosa or those with medical problems and therefore high surgical risks (8). Chemoprevention is an appealing non-surgical strategy that has earned serious consideration as a potential means of controlling cancer incidence. It was first defined by Sporn (9) in 1976 as: " the use of natural, synthetic or biologic chemical agents to reverse, suppress, or prevent the carcinogenic progression".

Currently more than 400 potential chemopreventive agents are under investigation including vitamins like vitamin E and A, carotenoids, folic acid, tea and COX 2 inhibitors (10, 11). Natural products represent a promising group of chemopreventive compounds due to their decreased dose-limiting toxicity profiles compared to the limitations of other chemotherapeutic agents. Phenolic and sulfur-containing compounds are two major classes of these natural chemopreventive compounds (12). Phenolic compounds are widely distributed in plants, such as green tea and soybean. Sulfur-containing compounds can be found in broccoli, turnips, watercress, and garlic (12).

Garlic (Allium sativum) is a vegetable that belongs to the Allium class of bulb-shaped plants, it contains high levels of sulfur containing compounds known as organosulfur compounds (OSCs), to which many of the beneficial effects of garlic have been attributed. Garlic has been found to have protective effects against coronary thrombosis (13),

atherosclerosis (14), as well as vascular disorders (15). It also has antifungal, antiparasitic, antibacterial (16) and most importantly anticancer functions (17).

The anticancer properties of garlic have been recognized for centuries. Many epidemiologic studies have surveyed the effect of garlic on cancers of the stomach, colon, head and neck, lung, breast and prostate, they concluded that there is a preventative effect of garlic consumption on stomach and colorectal cancer with inconclusive results about other types of cancer (18, 19). Garlic OSCs have been shown to inhibit proliferation and induce apoptosis of cancer cells both in culture and in mouse xenograft models (20).

The mechanism is not fully understood, but several modes of action have been proposed. These include their effect on drug metabolizing enzymes, antioxidant properties and tumor growth inhibition. Garlic OSCs may aid in cancer prevention by shifting the balance from a tumor-mediated pro-inflammatory to a host-mediated anti-tumor medium which may stimulate the immune system to eradicate an emerging tumor (21).

A variety of garlic preparations have been studied for their effects on cancer prevention. One of the better known garlic preparations is aged garlic extract (AGE). It is formed during garlic aging for up to 20 months in ethanol. During this process unstable compounds are converted into more stable compounds. The most abundant organosulfur compound in AGE is S-allylcysteine (22).

S-Allylcysteine (SAC) is a stable compound that remains unaltered in AGE for up to 2 years (23). It has been found to retard the growth of chemically induced and transplantable tumors in several animal models (24, 25).

To the best of our knowledge no attempts have been carried out in English literature to assess the effect of topical application of SAC in the management of oral dysplastic potentially malignant disorders, thus considering the numerous beneficial properties of this compound, it's worthy to shed further light on its efficiency as a novel mode of therapy for oral mucosal dysplasia.

MATERIALS AND METHOD

The research protocol was approved by the Ethics Committee of the Faculty of Dentistry, Alexandria University. Ten patients were selected from the outpatient clinic of the Department of Oral Medicine, Periodontology, Oral Diagnosis and Radiology, Faculty of Dentistry, Alexandria University. All selected cases had intra-oral red and/or white lesions. Then they were histopathologically confirmed to exhibit dysplastic changes (26). Two patients didn't complete the study period and thus they were eliminated from the study.

Exclusion criteria included patients with systemic diseases, those receiving any medications, pregnant or lactating women, patients manifesting any extra-oral lesions along with patients exhibiting carcinoma in situ or invasive carcinoma as revealed histopathologically. Patients who received prior treatment for their oral lesions in the 3 months' period preceding the beginning of the study were also excluded.

An informed consent was obtained from each patient after they were provided with detailed information about the study regarding the biopsies and photographs needed before and after therapy.

All lesions were photographed and their sizes were measured using a plastic ruler. The incisional biopsies were obtained from the most representative area of the lesion. The biopsied tissue was then fixed in 10% neutral buffered formalin and sent to the laboratory of the Oral Pathology department, Faculty of Dentistry, Alexandria University, where it was processed, embedded in paraffin wax and stained with Hematoxylin and Eosin for histologic grading of the dysplastic lesions (27).

S-Allylcysteine (Tokyo Chemical Industry, Japan) was mixed with Orabase at a concentration of 5mg SAC/1gm Orabase at the department of Pharmaceutics, Faculty of Pharmacy, Alexandria University.

Oral hygiene measures were performed to all the enrolled patients and all the potential oral irritants were eliminated. Smokers were instructed to stop their smoking habit. All patients were instructed to topically apply the prepared drug on the lesion in question 3 times daily for a period of 1 month. Patients were instructed to wait at least twenty minutes after application of the drug before eating or drinking to ensure adequate time for drug absorption by the tissues. All patients were followed up for any allergic or adverse reactions to the drug and to evaluate the lesions' response

The response to therapy was evaluated clinically and histopathologically according to the reduction in size, pain score if present and degree of dysplasia. The pain score used was the Numeric Rating Scale (NRS) (28). Clinical and histopathological examination was performed at baseline, at the end of the therapy (1 month) and 1 and 2 months after termination of the study.

STATISTICAL ANALYSIS:

Descriptive statistics was conducted. Sizes of the lesions before and after treatment were compared using Wilcoxon signed ranks test.

RESULTS

This study was carried out to evaluate the effect of topical application of S-allylcysteine (SAC) on ten patients with dysplastic oral potentially malignant disorders.

I- Clinical data of the enrolled patients

Topical application of SAC was well tolerated by all the patients. No adverse reactions or hypersensitivity reactions were reported or clinically detected. However, the strong garlic flavor of the drug was found to be offensive by some patients.

Table (1) shows the clinical data of the studied patients regarding gender and age. The mean age of the subjects was found to be 47.88 ± 6.01 years.

Table (1): Distribution of the studied cases according to demographic data (n = 8)

	No.	%
Sex		
Male	6	75.0
Female	2	25.0
Age (years)		
≤50	4	50.0
>50	4	50.0
Min. – Max.	40.0 – 57.0	
Mean ± SD.	47.88 ± 6.01	
Median	48.50	

II- Clinical results regarding NRS

Patients with leukoplakia did not experience any pain or burning sensation and so they were given a NRS score 0 before and after treatment. However, the two lichen planus patients were suffering from severe pain prior to the study period. The NRS at baseline score for both cases was 8. After termination of the treatment period, the NRS score decreased to 4 in one patient and to 3 in the other patient. After 2 and 3 months the NRS increased to 5 in the first case and 6 in the second case, nevertheless it was still lower than the baseline NRS score by at least 2 points

III- Clinical results regarding the size of the lesions

Table (2) and figure (1) show the size of the lesions at baseline and at various periods of follow up. At baseline the surface area of the lesions ranged between 144 mm^2 and 800 mm^2 with a mean of 344.9 ± 219 mm^2 and a median of 302.5

After termination of the therapeutic phase (after one month), there was a non-significant decrease in the lesions' sizes. The sizes ranged between 96 mm^2 and 720 mm^2 with a mean of 286.1 ± 229.1 mm^2 and a median of 250.0

After two months, the size of the lesions ranged between 0 mm^2 and 720 mm^2 with a mean of 295.8 ± 257.5 mm^2 and a median of 250.0. The decrease in the lesion size was also not statistically significant in comparison to the baseline values.

At the end of the study period after three months, the lesions sizes were found to be non-significantly less than that at baseline. It was ranging between 0 mm^2 and 540 mm^2 with a mean of 249.1 ± 201.6 mm^2 and a median of 250.0

Table (2): Comparison between the different periods according to clinical results regarding the size of the lesions (n= 8)

	Pre-operative	Post - operative		
		1 month	2 months	3 months
The size of the lesions				
Min. – Max.	144.0 – 800.0	30.0 – 720.0	0.0 – 720.0	0.0 – 540.0
Mean ± SD.	344.9 ± 219.4	286.1 ± 229.1	295.8 ± 257.5	249.1 ± 201.6
Median	302.5	250.0	250.0	250.0
% of change		**↓15.06**	**↓15.05**	**↓26.18**
p_{pre}		0.249	0.612	0.173
Sig. bet. periods		p_1= 1.000, p_2= 0.068, p_3= 0.068		

Sig between the different periods was done using Wilcoxon signed ranks test.

p_{Pre}: p value for comparing between pre-operative and each other period.

p_1: p value for comparing between 1 month and 2 months

p_2: p value for comparing between 1 month and 3 months

p_3: p value for comparing between 2 months and 3 month

statistically significant at p≤ 0.05

IV- Histopathological results

A total of ten patients (six males and two females) with oral dysplastic potentially malignant disorders were included in this study. Eight cases were diagnosed histologically as leukoplakia and two cases as erosive lichen planus. Two of the leukoplakia patients did not continue through the entire study time and were excluded from the results. The age of the diagnosed cases ranged between 40 and 57 years with a mean age of 49.83 years for men and 48.75 years for women.

Clinically all leukoplakia patients were smokers and showed white patches adherent to the underlying tissues, at the anterior part of the buccal mucosa (two cases), posterior part of the buccal mucosa (two cases) and at the palate (two cases).

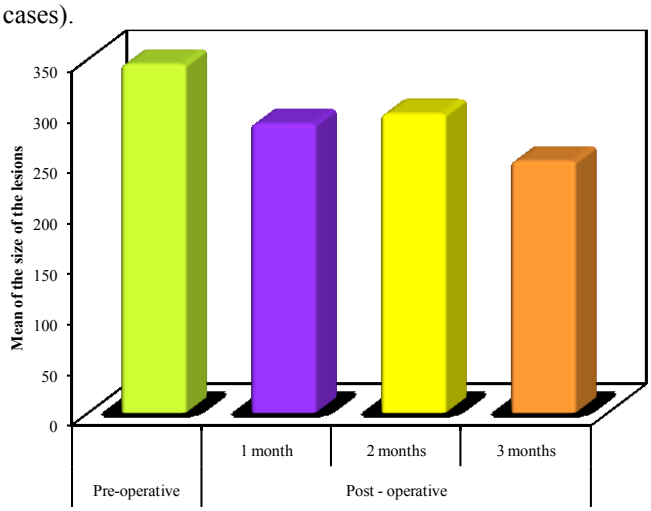

Figure (1): Comparison between the different periods according to clinical results regarding the size of the lesions (n= 8)

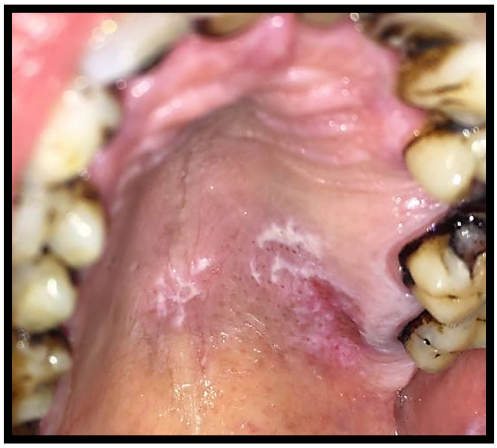

Figure (2): A case of leukoplakia in the posterior part of the palate at baseline before start of treatment. The red area represents the healing site of the pre-treatment biopsy.

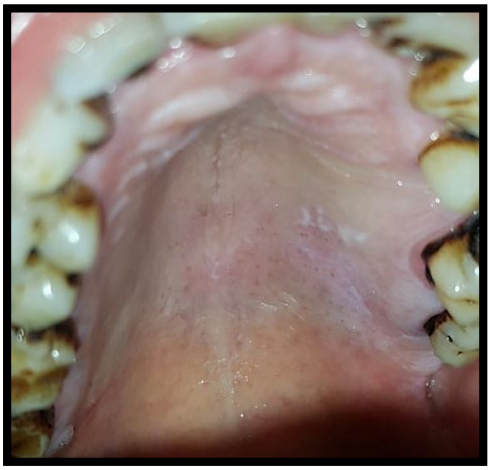

Figure (3): The same case with detectable clinical improvement after termination of the treatment period (after one month)

Microscopically oral leukoplakia of cases included in this study showed hyperkeratosis, acanthosis of the epithelium with various degrees of chronic inflammatory infiltrates in the lamina propria or connective tissue. Various degrees of epithelial dysplasia were detected as loss of polarity of basal

cells, increased nuclear cytoplasmic ratio, irregular stratification, cellular and nuclear pleomorphism and nuclear pleomorphism. Two cases of leukoplakia showed moderate dysplastic changes occupying two-thirds of the epithelial thickness, while the remaining four cases showed only mild dysplastic changes related to one-thirds of the epithelial thickness.

Cases of erosive lichen planus showed clinical ulcerative lesion with reddish white inflamed buccal mucosa. Histopathologically, lichenification of the basement membrane layer followed by a marked layer of lymphocytic infiltrate immediately underlying the epithelium was noted. The hyperplastic epithelial layers, acanthosis with variable thickness of the spinous layer and variable degrees of ortho or para-keratosis were also seen in the examined four cases.

One case of erosive lichen planus showed moderate epithelial dysplasia as hyperchromatism, pleomorphism, increase nuclear cytoplasmic ratio and discontinuity of the basal cell layer in some areas. Basement membrane lichenification was also present. The other case of erosive lichen planus showed mild epithelial dysplasia as pleomorphism and hyperchromatism conformed to the lower one-third of surface epithelium.

Patients received topical application of S-Allylcysteine three times per day for one month. Biopsies were repeated four weeks (one month) following treatment. The histologic response to treatment was assessed according to the following criteria, Complete response was defined as complete reversal to non-dysplastic squamous epithelium; Partial response (PR), regression of dysplasia to a lower grade; no response, persistence of pretreatment histologic grade; and Progressive response (PD), progression in grade of dysplasia or to invasive cancer.

Complete response was observed in four leukoplakia patients and two lichen planus patients where the mild and moderate dysplastic changes showed histologic remission of dysplasia. However, two leukoplakia cases showed progression in the grade of dysplasia from mild to moderate.

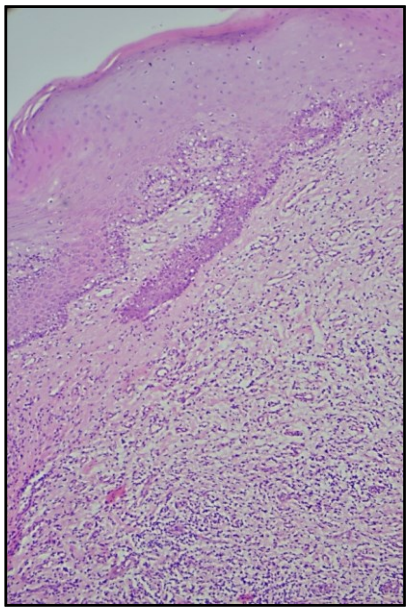

Figure (4): A photomicrograph of the previous clinical case of leukoplakia before the start of treatment showing irregular stratification with mild epithelial dysplasia as pleomorphism, hyperchromatism and loss of polarity of the basal cell layer can be detected. Diffuse infiltration of the connective tissue with chronic inflammatory cells are also noted (H and E ×100)

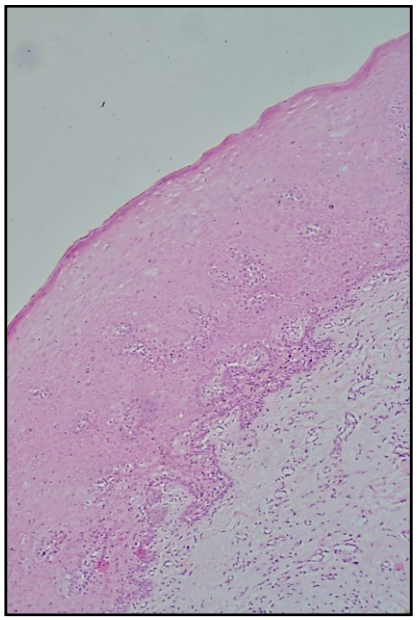

Figure (5): A photomicrograph of the previous case of leukoplakia after treatment with S-Allylcysteine showing the well stratification of the epithelial layers and absence of dysplastic changes along with the absence of the inflammatory cells in the connective tissue (H and E ×100)

DISCUSSION

In the current study, patients with systemic diseases, those receiving any systemic medications or displaying any extra-oral lesions were excluded from the study to decrease the number of confounding factors that may interfere with the results of the study. However, we could not exclude smoking patients due to the strong association between tobacco consumption and dysplastic oral potentially malignant lesions especially oral leukoplakia (29). Nevertheless, those patients were advised complete abstinence from tobacco to lower the risk of disease progression.

All possible oral irritants that may exacerbate the lesions like sharp cusps, rough dental restorations or ill-fitted dentures were identified and eliminated from the studied groups. In cases of white non-scrappable lesions, the lesions were followed up for at least 2 weeks following trauma elimination before taking the diagnostic biopsy to make sure the lesions persisted and exclude frictional keratosis (30).

The present study evaluates the clinicopathological response of some oral dysplastic potentially malignant disorders to the topical application of SAC. It involved 10 patients having OPMDs exhibiting mild to moderate dysplastic changes. Eight of them had oral leukoplakia and two had oral erosive lichen planus. Two of the patients with oral leukoplakia failed to complete the entire study period. The age of the patients ranged between 40 and 57 years. All leukoplakia patients were males while the 2 lichen planus patients were females. This is consistent with many other studies that found leukoplakia to be more dominant in males above the age of forty and lichen planus to be more common in middle aged females (31, 32) .

In the present work, each subject was assessed clinically and histopathologically according to three parameters: the size of the primary lesion, the pain score if present and the degree of dysplasia.

The pain score used in the present work was the numeric rating scale (NRS) as it is considered to be one of the simplest and most common scales used in pain interpretation (28) .Patients with leukoplakia did not experience any pain or burning sensation. Most of these patients were not even aware of the lesion's presence and so they were given a NRS score 0 before and after treatment. However, the two lichen planus patients were suffering from severe pain prior to the study period. They both noted a downward shift in the NRS score throughout the treatment phase. A possible explanation for the decrease in the NRS score noted in our results is the ability of SAC to reduce levels of COX-2 and subsequently prostaglandins (PGs) which might have resulted in reduction of pain hypersensitivity (33).

When comparing the size of the lesions before and at the various periods of follow up, a slight decrease in the size was noted but it was not statistically significant. However, the histopathological evidence of improvement was very remarkable as proven by the decrease or even total elimination of the signs of dysplasia.

Concerning the lichen planus patients, although all of the treated cases exhibited complete resolution of the dysplastic changes histopathologically, the clinical improvement fell short of the level of histopathological progress. This was clearly demonstrated by the persistence of the erosions despite elimination of dysplasia. Therefore, it's of interest to point out that although SAC led to elimination of the dysplasia, it was not clinically curative and it failed to improve the chronic behavior of LP. These results lie in accordance with a study conducted by Laeijendecker, et al., which compared the efficacy of topical tacrolimus ointment with triamcinolone acetonide ointment in 40 patients with oral lichen planus. The author noted that of the 27 patients who showed clinical improvement following therapy, relapses occurred in 20 patients within 3–9 weeks after cessation of treatment (34).

As for the leukoplakia patients, the clinical progress was more in line with the histological evidence of elimination of dysplasia. Most of the cases that exhibited complete resolution of dysplasia histologically demonstrated marked decrease in the lesions' size clinically. At the end of the treatment schedule one patient demonstrated complete lesion disappearance and two patients showed considerable decrease in the lesions' size. Also clinical signs of lesion deterioration noted in two of the leukoplakia cases were histopathologically associated with progression in the degree of dysplasia. However, the lesion remained the same in one patient in spite of complete elimination of dysplasia histopathologically.

The histological results illustrated in the current study substantiate the anticarcinogenic activity of SAC reported by many other authors. This chemopreventive effect of SAC may be attributed to its antiproliferative activity. In a study performed by Kevin et al. (35) SAC was found to have antiproliferative effect on hepatocellular carcinoma (HCC) cells. It was found to significantly induce apoptosis and necrosis of HCC cells in a dose-dependent manner through activation of cleaved caspase-3 and down-regulation of anti-apoptotic proteins like Bcl-2.

The anticancerous effect of SAC demonstrated in this study may also be mediated through its anti-oxidant activity. A study conducted by Hsu et al. has proven the effect of SAC on the induction of anti-oxidant enzymes like catalase and glutathione peroxidase (36). Dairam et al. (37) also proved SAC to act as chelating agents as it possesses the property of chelating metallic ions like Fe2+, Fe3+ and Cu2 in a concentration-dependent manner.

Nevertheless, one cannot deny the effect of eliminating or lowering tobacco use on the positive results detected in these patients. Most of the patients who exhibited improvement in their lesions stopped or cut down on their tobacco use after the first diagnosis of dysplasia. Elimination of chronic irritation by tobacco plays a major role in the improvement displayed in our cases. This lies in accordance with a case-control study performed by Morse et al. to evaluate the association between oral epithelial dysplasia (OED) and the use of smoking tobacco and alcoholic beverages (38). The authors found the risk of OED to increase with increasing levels of smoking and declined following smoking cessation.

Concerning the two leukoplakia patients displaying histopathological signs of disease progression, it is worth mentioning that these were the same two patients who presented with an increase in the lesion's size clinically following termination of the treatment period. One of these patients was found to completely neglect the instructions given about cessation of smoking. The patient continued to smoke heavily throughout the treatment and follow up phases and so the persistence of tissue irritation by tobacco represents a reasonable explanation for the lesion's deterioration (38). The other patient however, reported stoppage of the smoking habit completely by the end of the treatment period. We speculate that the unexpected worsening in the degree of dysplasia detected in this patient after termination of the therapeutic phase may be explained by persisting clonal expansion as it's commonly believed that cancerous progression involves successive waves of clonal expansions within the lesion. This uncommon response to therapy was reported in a clinical trial conducted by Mao et al. on 7 patients with oral dysplastic lesions (39).

The patients were treated with a combination of 13-cis-retinoic acid, interferon α and α-tocopherol for 12 months. Two of the treated patients developed invasive cancinoma and two developed new lesions. The author hypothesized that the reason behind the progression of the dysplastic lesions is the persistence of some of the clonal genetic abnormalities that were resistant to the therapeutic agents.

In conclusion, it appears from the present study that S-allylcysteine plays an important role in the management of dysplasia associated with oral potentially malignant disorders.

CONCLUSION

1- S-Allylcysteine does not cause adverse or allergic reactions.

2- Topical application of S-Allylcysteine is more beneficial on the histological level than the clinical level.

3- Topically applied S-Allylcysteine is a promising chemopreventive agent that can be considered for the management of dysplasia associated with oral potentially malignant disorders.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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